SHORT COMMUNICATION

Seasonal Changes in the Lipid Classes and Fatty Acid Compositions of Hake (*Merluccius hubbsi*) Liver Oil

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ABSTRACT: The lipid content, lipid class composition, and fatty acid composition of hake (*Merluccius hubbsi*) liver oil was studied for a whole year. The lipid content of the liver ranged from 41 to 60%. Triacylglycerols were the main lipid class present in the oil, with a mean value of 80%. Docosahexaenoic acid was the main polyunsaturated fatty acid during the year and was twice that of the eicosapentaenoic acid content. The composition of hake liver oil makes it useful as a raw material for encapsulated pharmaceutical products.
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KEY WORDS: Docosahexaenoic acid, eicosapentaenoic acid, fatty acid composition, fish liver oil, hake liver oil, lipid class composition, *Merluccius hubbsi*, seasonal changes.

Fish oil production in Uruguay is limited to a sub-product of the fish meal industry, with an estimated annual production of 500 ton. However, the Southwest Atlantic hake (*Merluccius hubbsi*), the main fishing resource of Uruguay, offers an excellent possibility for fish oil production as a result of some advantages. As in other lean fishes, the livers are the depot lipid storage organ, and the oil can be easily extracted by centrifugation of the livers with good yield without need for flammable solvents (1). Attempts to concentrate the polyunsaturated fatty acids as urea adducts gave high yields (2) and in spite of lower stability toward oxidation in comparison to other fish oils (3), *tert*-butyl hydroquinone (TBHQ) at a 200-ppm level increased oxidative stability by six times (4).

Hake is a migratory fish and has two spawning periods, one in autumn and the other in spring (5). These biological events lead to remarkable seasonal changes in the energetic status, which in time produce changes in the lipid composition of muscle (6) and gonads (7).

In this work, the seasonal changes in the lipid content, lipid classes, and fatty acid composition of the oil extracted from hake livers in five different months during the year 1992 is reported.

EXPERIMENTAL PROCEDURES

Five batches of Southwest Atlantic hake, each comprised of 10 fish, were obtained from the Argentinean–Uruguayan Common Fishing Zone in February, April, July, September, and December 1992. The mean length of the specimens was 48 cm (range 42–54 cm), and the mean weight was 800 g (range 588–1075 g). From each specimen in each sampling month, a portion of the liver was excised and homogenized in a food processor and stored under nitrogen atmosphere at –20°C prior to sampling for chemical analysis.

All chemical analyses were performed (on composite samples) in duplicate. Total lipids were extracted and purified by the Folch *et al.* procedure (8) and quantitated gravimetrically. Lipids were fractionated on silica gel G plates, 0.25-mm thickness (Machery-Nagel, Düren, Germany) with the solvent mixture petroleum ether (b.p. 60–80°C)/diethyl ether/acetic acid (80:20:1, vol/vol/vol). Quantitation was carried out by direct densitometric measurements on the plate stained with 10% $CuSO_4$ (wt/vol)/8% H_3PO_4 (vol/vol) (9) and heated at 140°C for 20 min. A thin-layer chromatographic scanner (Shimadzu CS-9000, Kyoto, Japan), operated in the reflectance mode at 500 nm, was used for lipid class quantitation. Coefficient of variation for the different lipid classes was less than 2%. Fatty acid composition was assessed by gas chromatography of their methyl esters, obtained according to AOCS method Ce 1b-89 (10) and analyzed in a Hewlett-Packard 5840A gas chromatograph (Palo Alto, CA). A stainless-steel column, 3 m length and 3 mm i.d., packed with 10% SP-2330 in Supelcoport 100/200 mesh AW (Supelco Inc., Bellefonte, PA), was used. The temperature program was 12 min at 185°C, heated to 230°C at a rate of 2°C/min, and then constant temperature (230°C). Coefficient of variation for this analysis was less than 5% for those fatty acids in percentages greater than 2%.

RESULTS AND DISCUSSION

Large seasonal changes in the lipid content and in the lipid class composition were observed (Table 1). The lipid content had its highest value in February, coincidentally with the top of feeding activity (5). The content of triacylglycerols varied according to the lipid content, a result expected, considering that the liver is the fat storage organ of hake (11). The steryl ester content of the oil showed a progressive increase from February (summer) to September (spring), and decreased drastically after that, coinci-

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TABLE 1
Seasonal Changes in the Lipid Content (g/100 g liver) and Lipid Class Composition (g/100 g oil) of Hake Liver Throughout the Year 1992

	February	April	July	September	December	Mean
Lipid content	60.0	50.7	48.7	41.0	45.9	48.8
Lipid class						
Phospholipids	1.3	5.1	3.1	3.1	3.5	3.2
Diacylglycerols	2.2	4.2	1.6	1.3	1.9	2.2
Sterols	1.7	2.6	0.2	0.8	2.8	1.6
Steryl esters	2.7	6.6	15.9	16.9	trace	8.4
Free fatty acids	3.8	6.5	3.6	3.8	4.5	4.4
Triacylglycerols	88.2	75.0	75.0	74.1	87.4	79.9

dental with the spring spawn. In accordance, the sterol content showed an inverse relationship to that of the steryl ester content. Steryl esters are considered the transport molecules of sterols (12), and the results obtained suggest that sterols were mobilized from the liver to the gonads for spawn.

Phospholipid content was low in all sampling months. This low content and the high concentration of triacylglycerols make the liver a soft organ, explaining the ease with which the oil is extracted by centrifugation (1). The highest free fatty acid content was 6.5% during the year, and this figure is almost reduced to 0 if aqueous NaOH is added to the livers prior to centrifugation (1).

Palmitic (16:0), oleic (18:1), eicosenoic (20:1), docosenoic (22:1), eicosapentaenoic (20:5), and docosahexaenoic (22:6) acids were the principal fatty acids present in the oil (Table 2). It is remarkable that 20:5 < 22:6 throughout the year, a fact characteristic of long-lived and southern fishes (13).

Also important is the presence of 20:1 and 22:1 fatty acids. These fatty acids are known to be of exogenous origin (14). Ackman *et al*. (15) suggested that, if these acids are discounted from the composition, the resulting calculated oil should be similar to menhaden oil, which lacks these acids, and might represent a basic fish oil composition. To prove this hypothesis with hake liver oil, the fatty acid composition was calculated by discounting the 20:1 and 22:1 content from the mean composition, and comparing it to menhaden oil that was analyzed under the same conditions. The calculated fatty acid composition gave (ignoring minor components) 26.2% saturates, 30.7% monounsaturated and 34.5% polyunsaturated, and closely resembled that of the menhaden composition, in which saturated, monounsaturated, and polyunsaturated fatty acids comprised 27.7, 27.6, and 31.5%, respectively.

TABLE 2
Seasonal Changes in the Fatty Acid Composition (in wt%) of Hake Liver Oil Throughout the Year 1992

Fatty acid	February	April	July	September	December	Mean
14:0	3.8	4.2	3.5	3.6	2.6	3.5
16:0	16.4	15.0	15.9	17.3	16.5	16.2
18:0	1.9	1.7	2.2	1.9	2.5	2.0
Total SAT[a]	22.1	20.9	21.6	22.8	21.6	21.7
16:1	6.3	7.0	6.3	7.8	8.5	7.2
18:1	15.6	15.4	20.4	20.8	24.6	19.4
20:1	6.3	5.5	8.6	8.6	6.5	7.1
22:1	8.6	5.9	6.7	7.5	5.0	6.7
Total MUFA[a]	36.8	33.8	42.0	44.7	44.6	40.4
18:2	2.0	2.8	2.2	2.0	2.3	2.3
18:4	3.6	5.6	3.4	2.4	3.1	3.6
20:4	1.5	2.0	1.7	1.1	1.5	1.6
20:5	8.4	7.1	5.0	6.0	7.9	6.9
22:6	15.2	17.6	12.7	13.1	13.2	14.4
Total PUFA[a]	30.7	35.1	25.0	24.6	28.0	28.8

[a]Ignoring minor components (< 1%); SAT, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

In conclusion, hake liver is a rich source of triglyceride oil that is high in polyunsaturated fatty acids, specially in 22:6 fatty acid. The fatty acid composition indicated that this oil would be a good raw material for fish oil capsules.

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